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SYNTHESIS AND SCREENING OF NEW ANTIHYPERTENSIVE DRUGS
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SYNTHESIS AND SCREENING OF NEW ANTIMALARIAL DRUGS

ANNUAL REPORT

M.M. DHAR

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Central Drug Research Institute
Lucknow-226001 (India)

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The PL480 project has completed its second year and rhesus monkey models for radical curative and causal prophylactic testing have been validated. Construction of a large multistory primate colony building has been completed. The activities of 13 WRAIR and 3 CDRI compounds were evaluated in the rhesus monkey model with five compounds found to be more active than the primaquine controls. Radical curative properties of 11 compounds (5 WRAIR and 6 CDRI) were evaluated with 5 compounds found to be more active than primaquine controls.																	
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PROGRESS REPORT

The protocol of "Programme Plan for Institution of Research Collaboration" jointly developed by WRAIR, Washington and C.D.R.I., Lucknow, has been followed and two test systems namely (1) Radical Curative Test for development of new antirelapse compounds and (2) Prophylactic test for antisporeozoite causal prophylactic activity, have been established at the Central Drug Research Institute. The standardization of both these tests is over, and the results of screening tests have been validated and revalidated using standard test drug primaquine. The test systems are ready for large-scale evaluation and pre-clinical efficacy trials of potential radical curative and causal prophylactic antimalarial drugs being synthesized and developed by the US Army Antimalarial Drug Programme and by C.D.R.I. Screening of potential compounds in both the test systems has been started.

A. Rhesus Radical Curative Test

The major task for the project was to establish reproducible seven day test system for radical curative activity using sporozoite induced Plasmodium cynomolgi B-Rhesus monkey model and ensure the reproducibility of the results on standard drug primaquine obtained at C.D.R.I. with those of AFRIMS. Work on first three phases: (1) Standardization of sporozoite infection, (2) Chloroquine dose (as blood schizontocide) and (3) Primaquine dose for radical curative activity (validation and revalidation), is over and the work on IVth phase i.e. Evaluation of reference drugs WR 242511 and WR 249252, and some candidate drugs, has been undertaken.



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Phase I: Standardization of Sporozoite Infection

a). Insectary

Technology for production of sporozoites of P. cynomolgi B for successful inoculation of 20-25 monkeys at a time has been established at C.D.R.I., and the new Insectorium has been made fully operational. Mass rearing of A. stephensi (NICD strain) has been maintained and an average of 2000-4000 eggs are procured daily. This ensures the availability of all the four larval instars at all times for obtaining 2-3 day old mosquitoes for infective blood meal on the gametocyte carrying monkeys. The insectary maintains on the average 20,000-30,000 larval/pupal stages and 1500-2000 adults emerge daily. The period from egg to 4th larval instar ranges between 10-14 days, and pupal once formed hatch into adults within 36-48 hrs.

b). Infection of mosquitoes

Maximum infectivity is obtained when the mosquitoes are fed during the secondary peak parasitaemia and an average of 30-80 oocysts are observed/gut on day 7.

c). Estimation and harvesting sporozoites from infected mosquitoes

Following discussions with Major Richard Andre from AFRIMS and COL Davidson, the method of sporozoite estimation and harvesting has been improved. For accurate counting, slide is examined for consecutive 100 oil immersion fields from edge to edge of the FA slide. Harvesting method has been slightly modified which enables us to obtain higher yield of sporozoites for

inoculation of monkeys. After grinding the mosquitoes, the suspension is centrifuged at 1000 rpm for 15 seconds at 4°C. The sediment, after removing the supernatant, is resuspended in 2-5 ml of serum saline mixture and recentrifuged at 1000 rpm for 15 seconds. This supernatant is added to the first supernatant and then processed for inoculation of monkeys.

d). Serial cyclic passage of sporozoite induced infection in rhesus monkeys

Since the inception of the project, 17 serial sporozoite passages of P. cynomolgi B have been carried out and details of passages 10-17 are given in Table 1.

Phase II. Chloroquine dose

Experiments on determination of 100% curative dose of chloroquine against blood induced P. cynomolgi B in rhesus monkeys inoculated with 1×10^5 parasitized RBC by i/v route were completed last year and results showed that 3.0 mg/kg (base) x 7 days was curative dose. The same dose was found to be effective when 5 monkeys which showed recrudescence at 1.0 mg/kg were retreated.

Revalidation of curative dose of chloroquine: Studies on revalidation of chloroquine dose were also reported last year and 3.0 mg/kg x 7 days was again found to be consistently curative against blood induced infections. So this phase of study is complete.

As reported previously, although 3.0 mg/kg chloroquine(base) was curative, we have preferred to use 5 mg/kg chloroquine as the curative dose for our radical curative test studies.

Phase III. Primaquine Dose

The validation of radical curative dose of primaquine and revalidation studies reported last year showed that 1.0 mg/kg x 7 days was curative as shown by absence of any relapse beyond 90 days after the end of treatment. The lowest dose found active was 0.56 mg/kg x 7 days in 2 monkeys (No. 1677, 1679). A dose of 0.316 mg/kg was non-curative as both the monkeys (No. 1733, 1734) at this dose relapsed on day 18 and 28 respectively.

During this year, radical curative efficacy of 0.56 mg/kg dose of primaquine (x7 days) has been reevaluated and up to 70 days of observation, this dose has again been found to be curative in four monkeys (No. 2753, 2754, 2755, 2757). Further observations are continuing (Table 2). Besides a dose of 1 mg/kg primaquine has again been found to be curative in two monkeys (No. 2704, 2705).

In our radical curative tests, a dose of 0.56 mg/kg seems to be curative; however, we prefer to use a dose of 1.0 mg/kg x 7 day as our standard radical curative dose.

Phase IV: Candidate Drugs

Tests on the radical curative activity of the following four primaquine putative metabolites initiated during the previous year have been completed.

1) RCGJM 53. (5-hydroxy primaquine)

This compound was tested at 2.0 mg/kg dose in two monkeys (No. 2356, 2357) and both of them were radically cured (Table 3).

2) RCGJM 33: 5,6-dimethoxy-3-aminoquinoline

This compound was inactive at 1.58 mg/kg x 7 days in two monkeys (No. 2337, 2338). Both the monkeys relapsed on day 15 and 16 respectively.

3) CDRI 3]/472 (6-methoxy-8-aminoquinoline)

This compound was inactive at 135 mg/kg x 7 days in two monkeys (No. 2235, 2236). Both the monkeys relapsed on day 17 and 14 respectively.

4) RCGJM 161 (6-hydroxy-8-aminoquinoline)

This compound was inactive at 2.36 mg/kg x 7 days in two monkeys (No. 2237, 2238). Both the monkeys relapsed on day 17.

Besides the above primaquine metabolites, three derivatives of primaquine (namely N-galacto-sido-primaquine (CDRI 83/383), N-glucosido-primaquine (CDRI 83/382) and N-mannosido-primaquine (CDRI 84/136) have been tested for their radical curative activity.

5) CDRI 83/383 (N-galactodiso-primaquine)

Two preparations of this compound (1. Amorphous, 2. Crystalline) have been tested for radical curative activity.

1. Amorphous preparation was tested in first experiment at 3.25 mg/kg x 7 days in two monkeys (No. 2279, 2280), at 1.62 mg/kg x 7 days in two monkeys (No. 2204, 2205), at 0.82 mg/kg in one monkey (No. 2203). All the five monkeys including the one at 0.82 mg/kg were cured. However, in the second experiment five monkeys (No. 2321, 2346, 2377, 2376, 2379) were tested at the above dose (0.82 mg/kg), and all the five monkeys relapsed on day 23, 24, 41, 53, and 16 respectively. The lower doses 0.51 mg/kg and 0.51 mg/kg tested in two monkeys each were inactive.

2. Crystalline preparation was tested at 0.82 mg/kg and 0.41 mg/kg x 7 days in two monkeys each. The dose of 0.82 mg/kg was curative in monkey No. 2361 and 2369 and the lower dose 0.41 mg/kg was inactive in two monkeys (No. 2367, 2368). The effective dose 0.82 mg/kg corresponds to 0.50 mg/kg primaquine base.

6) CDRI 83/382 (N-glucosido-primaquine)

This compound was tested in three experiments. A dose of 0.82 mg/kg x 7 days of N-glucoside-primaquine (containing 0.5 mg/kg primaquine base) was curative in 8 monkeys (expt. I - No. 2390, 2391, 2393, 2397, and 2398, and Expt. II No. 2535, 2536, and 2537). The lower dose 0.41 mg/kg was tested in 11 monkeys, out of which 3 monkeys (No. 2360, 2366, and 2449) were successfully cured and the remaining 8 monkeys relapsed between day 21-77. Three monkeys at 0.20 mg/kg also relapsed between day 14-22.

7) CDRI 84/136 (N-mannosido-primaquine)

This compound was tested at 1.62 mg/kg x 7 days in two monkeys (No. 2353 and 2289) and at 0.80 mg/kg in three monkeys (No. 2714, 2723, and 2724). Both these doses were curative. The lower dose of 0.80 mg/kg correspond to 0.50 mg/kg primaquine base.

8) CDRI 84/137 (N-glucosido-6-methoxy-8-amino-quinoline)

This compound was tested at 5.0 mg/kg in two monkeys (No. 2330 and 2283) and the monkeys relapsed on day 14 and 15.

9) CDRI-RCG9 (Bromoprimaquine)

This compound was tested at 3.16 mg/kg and 1.00 mg/kg x 7 days, in two monkeys at each dose level. Both the doses were inactive.

10). WR 242511.

The compound was tested in 1st experiment at 4 dose levels x 7 day (1.0 mg/kg; 0.316 mg/kg, 0.10 mg/kg and 0.0316 mg/kg dose levels in three monkeys each). Doses of 1.0, 0.316 and 0.10 mg/kg were fully curative. At 0.0316 mg/kg dose, however, two monkeys (No. 2321, 2382) were radically cured, while the 3rd monkey (No. 2384) showed a relapse on day 51.

In the second experiment, five monkeys were tested at each of the 3 dose levels (0.316 mg/kg; 0.10 mg/kg and 0.0316 mg/kg x 7 days). All the three dose levels were curative.

This compound was sent as reference for validation of our test system and our results show that the primaquine index of the compound seems to be more than 10.

This compound was tested at 4 dose levels x 7 days (1.0 mg/kg, 0.316 mg/kg, 0.10 mg/kg and 0.0316 mg/kg, in two monkeys at each dose). The compound was curative at 1.0 and 0.316 mg/kg dose levels. At 0.10 and 0.0316 mg/kg doses, the treated monkeys showed relapse; monkeys at 0.10 mg/kg relapse on days 23 and 60, and those at 0.0316 mg/kg relapsed on day 14 and 15.

This compound was also sent as reference for our tests. Revalidation of these results will be carried out.

B. Rhesus Prophyl-Actic Test

Studies on 9 day rhesus prophylactic test using sporozoite induced P. cynomolgi B proposed by Schmidt and the modified 3 day test proposed in our project for assessment of the causal prophylactic activity of the primaquine as standard drug are over.

Phase I. Sporozoite Infection

Standardization of the protocol for successful sporozoite induced infection of P. cynomolgi was reported last year. The same methodology has been employed during the current year and is given under Phase I Radical Cure test. Sporozoite inoculated monkeys become patent on day 8 or 9 as shown by examination of thick blood smears of the infected monkeys.

Phase II. Primaquine Dose

A. 9-Day Test

The test system for prophylactic test using sporozoite induced P. cynomolgi B has been made operational and primaquine dose required for curative action in standard 9-day test by oral route was reported in the last year's report. At 1.0 mg/kg dose of primaquine x 9 days, all three monkeys (No. 2105, 2107, and 2339) were cured. Primaquine was also given by intravenous route in 9 day test as given in previous years report and 2 mg/kg dose x 9 days was curative in four monkeys (No. 2339, 2340, 2231, and 2232), 1 mg/kg dose x 9 days was curative in five monkeys (Nos. 2110, 2281, 2282, 2283, 2284) while one monkey at this dose (No. 2109) became patent on day 28. Lower dose 0.5 mg/kg x 9 day was ineffective in two monkeys (No. 2103, 2112). Since the treatment in this test is given for 9 days, the test is unable to differentiate the ant sporozoite and tissue schizontocidal activity.

B. 3-Day Test

As proposed in the project protocol, the 3-day test system for causal prophylaxis has been standardized and five experiments (I to V) on primaquine dose for 3-day prophylactic test have been concluded (this includes expts. I to III reported previously). The purpose of this study was to delineate the true antisporeozoites activity of the test compound from its tissue schizontocidal action and, therefore, the treatment period was reduced from 9 days to 3 days (-1 day, 0 day, and +1 day). Besides the 3-day system has the advantage of reducing the drug administration period for compounds like primaquine which are likely to cause hepato- and haematological toxicity if administered for longer periods. The data summarized in the Tables, show that doses of 1.30 mg/kg, 1.78 mg/kg, 3.16 mg/kg, and 10.00 mg/kg were curative in 3-day test system against sporozoite induced infection in rhesus monkeys. At 1.00 mg/kg, 9 out of 16 monkeys were cured in 3-day test. Lower doses (0.316 mg/kg and 0.62 mg/kg) were ineffective).

The studies on 3-day test have shown that 11 monkeys at 1.78 mg/kg primaquine (base) dose put in 4 different experiments with sporozoites inoculation have shown curative action.

Phase III. Candidate Drugs

A. Nine-Day Test

Following the protocols developed for causal prophylactic activity of primaquine in 9-day test system (described above under Phase II), we have screened seven putative metabolites of

primaquine and two primaquine derivatives, the results of which are summarized below and the details were given in last year's report:

1) RCGJN 52 (5-methoxyprimaquine)

This compound showed causal prophylactic activity at 2.23 and 1.11 mg/kg dose x 9 days in two monkeys each.

2) RCGJM (5-hydroxyprimaquine)

The compound was toxic after 2 doses at 2.12 mg/kg after intravenous administration, but at 1.06 mg/kg dose x 9 days by oral route, the compound was inactive.

3) RCGJM 55 (5-hydroxy-6-desmethylprimaquine)

The compound was inactive in 2 monkeys at 2.01 mg/kg x 9 day dose by intravenous route.

4) CDRI 83/472

The compound was inactive in 2 monkeys at 1.35 mg/kg x 9 day dose by intravenous route.

5) RCGJM-33 (5,6-dimethoxy-8-aminoquinoline)

The compound was tested at 1.58 mg/kg by both intravenous/oral routes in the two monkeys each, and was found to be inactive.

6) RCGJM 162 (5-hydroxy-6-methoxy-8-aminoquinoline)

This compound at 2.94 mg/kg dose was toxic in two monkeys after intravenous administration whereas at 1.47 mg/kg dose x 9 days it was inactive in two monkeys by oral route.

8) CDRI 83/383 (N-galactosidoprimaquine)

This compound was tested in two monkeys each at three dose levels (3.25, 1.62, and 0.51 mg/kg x 9 days) by oral route and

it was found to be active at all the three dose levels. The revalidation of these data will be carried out.

The tests done so far show that this compound has a primaquine index of approximately 2 in 9-day causal prophylactic test. The lowest effective dose of 0.51 mg/kg is equimolar to 0.3 mg/kg primaquine base.

9) CDRI 83/382 (N-glucosidoprimaquine)

This compound was tested at three dose levels (four monkeys at 3.25, two monkeys at 1.62 and two monkeys at 0.51 mg/kg x 9 days) by oral route, and it was found to be active at all the three dose levels. The revalidation of these data will be carried out.

The tests done so far show that this compound has a primaquine index of approximately 2 in 9-day causal prophylactic test. The lowest effective dose of 0.51 mg/kg is equimolar to 0.3 mg/kg primaquine base.

B. Three Day Test

The results of the prophylactic activity of 4 putative primaquine metabolites described below (compounds 1-4) were reported in last years report and the data are summarized in this report.

(1) WR 6890 (6-hydroxy-8-aminoquinoline)

This compound was under test last year and the results are now complete and data are given in Table It was tested orally at 4 dose levels (10.0, 3.16, 1.00, 0.316 mg/kg x 3 days) in two monkeys each. The highest dose (10.0 mg/kg) has been found to be active.

(2) WR 15081 (6-methoxy-8-aminoquinoline)

This compound was tested orally at 4 dose levels (10.00, 3.16, 1.00, 0.316 mg/kg x 3 days) in two monkeys each, and was found to be inactive.

(3) WR 199507 (5-hydroxyprimaquine)

This compound was tested orally at 4 dose levels (10.00, 3.16, 1.00, 0.316 mg/kg x 3 days) in two monkeys each, and was found to be inactive.

(4) WR. 250016 (5-hydroxy-6-desmethyl-Primaquine)

This compound was tested orally at 4 dose levels (10.00, 3.16, 1.00, 0.316 mg/kg x 3 days) in two monkeys each, and was found to be inactive.

Five new compounds have been screened for the prophylactic activity in 3-day tests during the current year and the results are summarized below and details are given in Tables.

(5) WR 242511

The compound was tested orally in two experiments. In Expt. I, four dose levels (1.78, 1.00, 0.316, 0.10 mg/kg x 3 days) were tested by oral administration in two monkeys at each dose level. All four doses were found to be curative. In Expt. II, three dose levels were tested i.e. two monkeys at 0.316 mg/kg, five monkeys at 0.10 mg/kg, and two monkeys at 0.0316 mg/kg x 3 days. The doses of 0.316 and 0.10 mg/kg were consistently curative. However, the lowest dose (0.0316 mg/kg) was inactive.

(6) WR 225448

The compound was tested orally at 4 dose levels (1.78, 1.00, 0.316, and 0.10 mg/kg x 3 days) in two monkeys each. All the doses were curative. The revalidation of the data will be carried out in larger number of monkeys.

(7) WR 238605

The compound was tested orally at 4 dose levels (1.78, 1.00, 0.316, and 0.10 mg/kg x 3 days) in two monkeys each. The doses of 1.78, 1.00, and 0.316 mg/kg were found to be effective while one of the monkeys at 0.10 mg/kg dose became patent. The revalidation of this compound will be carried out in more number of monkeys.

(8) WR 249420

The compound is under test at 4 dose levels (1.78, 1.00, 0.316, and 0.10 mg/kg x 3 days) by oral route. Observations up to 40 days show the activity of this compound at all the doses tested in two monkeys each. Further observations are continuing.

(9) CDRI.RCG 9 (Bromoprimaquine)

The compound has been tested orally at two dose levels (3.16 and 1.00 mg/kg x 3 days) and it has shown activity in one of the two monkeys at 3.16 mg/kg dose, while the lower dose (1.00 mg/kg) was inactive.

CDRI PRIMATE ANTI-MALARIAL STUDY

Title: 1 Serial passages of sporozoite induced P. cynomolgi
E infection

Sporozoite Passage No.	Monkey Number	Date of Inoculation	Sporozoite Inoculum (I.V.)	Day of Patency
X	2242	20.2.84	0.64×10^6	9
	2325	5.3.84	0.78×10^6	9
	2337	5.3.84	0.75×10^6	9
XI	2354	27.3.84	1.1×10^6	8
	2355	27.3.84	1.1×10^6	8
	2542	27.4.84	0.66×10^6	9
XII	2569	23.5.84	0.76×10^6	9
XIII	2642	19.6.84	0.82×10^6	8
XIV	2644	30.7.84	0.82×10^6	8
XV	2775	7.9.84	0.68×10^6	9
	2803	7.9.84	0.68×10^6	9
XVI	2811	17.10.84	0.66×10^6	9
	2812	19.10.84	0.58×10^6	10
	2917	22.10.84	0.72×10^6	9
XVII	2963	4.12.84	0.66×10^6	9
	2964	4.12.84	0.66×10^6	9

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